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Influence of genetic milk protein variants on milk quality

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ABSTRACT

Due to substitution, elimination or addition of amino acids, different genetic milk protein variants vary in their molecular properties, i.e. net electrical charges, hydrophobicity and three-dimensional structure along the peptide chain. These differences influence the technological properties of the milk to a varying extent. Despite a lot of contradictory results over the years within this study field, it is generally agreed that the B variants of κ-casein and β-lactoglobulin are correlated to higher casein content, better curd firmness and higher cheese yield than the A variants. The influence of other genetic variants is also discussed in the paper.

KEY WORDS: casein, whey proteins, genetic polymorphism, technical properties, curd firmness

MOLECULAR BACKGROUND AND REACTIONS OF MILK PROTEINS

There is extensive literature on the influence of genetic polymorphism on the technological properties of milk. To understand the variation in results presented, it is important to know how different genetic variants of a certain protein vary in properties and also what happens in detail when different milk products are produced.

The caseins in milk, ≈80% of the protein content, are relatively small amphiphilic phospho-proteins with the unique property to bind high amounts of calcium without precipitating. The latter is due to formation of casein micelles, of which the structure not yet is fully understood, even though several models have been and are still discussed. The caseins are divided into α_{s1}-, α_{s2}-, β- and κ-casein (-CN) according to their electrical net charge and contain both hydrophobic and hydrophilic parts.

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The hydrophilic parts of α_{s1}-, α_{s2}- and β-CN bind calcium to different degrees, which in turn bind to calcium phosphate (CaP) nanoclusters. In the casein micelle model of Horne (1998, 2006) the caseins are bound to each other through both hydrophobic interaction and CaP-bridges. However, since κ-CN does not bind CaP, κ-CN acts as a chain terminator and stops further aggregation of casein molecules into the micelle (see overview in Figure 1). Thus, most of the κ-CN is therefore located on the surface of the micelle (generally agreed), where the hydrophilic and also negatively charged part (tail) of κ-CN sticks out and repels other micelles, resulting in an even distribution of casein micelles within the milk. The properties and localization of κ-CN is also the reason for the milk-clotting reaction due to loss of the hydrophilic and negatively charged surface of the micelle after treatment with rennet. The rennet (e.g., chymosin and pepsin) cuts the tails off the surface (Figure 1), which then changes into a hydrophobic surface, causing in turn the micelles to aggregate (clot). After cutting, stirring, heating, moulding, pressing and ripening, the milk gel is developed into cheese.

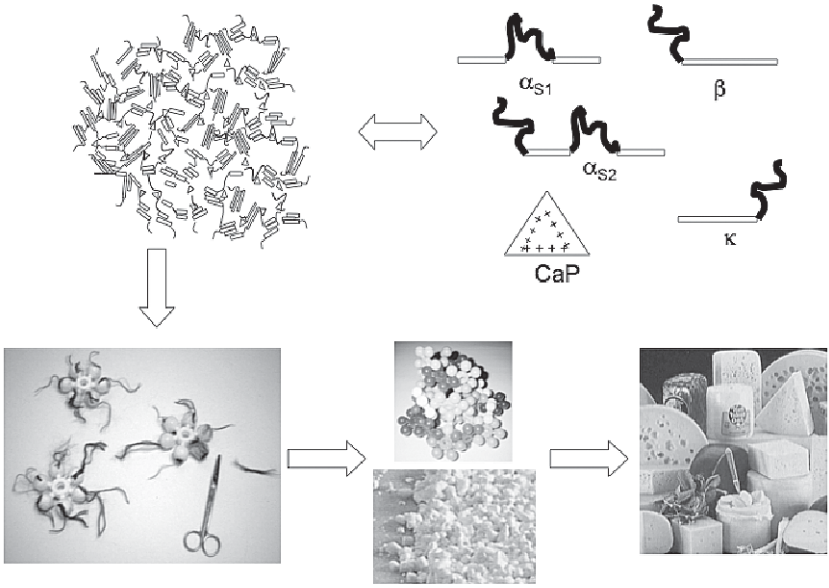

Figure 1. Overview of the milk-clotting reaction and cheese making

The κ-CN and α_{s2}-CN contain cysteine residues, which give rise to disulphide linkages between κ-CN molecules and within α_{s2}-CN molecules. The whey proteins, α-lactalbumin (-LA) and β-lactoglobulin (-LG), also contain disulphide bridges and in addition, β-LG also contain a free sulphide group (thiol, -SH). The thiol group is hidden within native β-LG molecules, but is exposed upon heating and reacts both with other whey proteins as well as κ-CN and α_{s2}-CN on

the casein micelle surface (see overview in Figure 2). This is taken into account in the production of fermented milks, where the heat treatment gives a better consistency and water holding capacity of the acidified milk (yoghurt) due to a layer of especially β-LG molecules on the micelle surface at heating; the longer the heating time, the thicker the layer. Without heating the acidifying of the milk only gives a granular precipitate of the caseins (Figure 2).

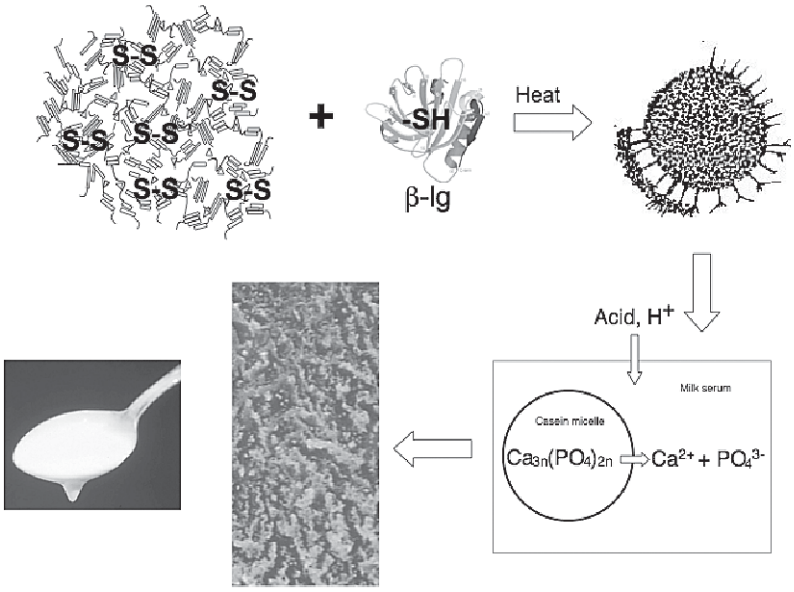

Figure 2. Overview of heat-induced reactions of milk proteins and production of fermented milk

GENETIC VARIANTS OF THE MILK PROTEINS

Since the first discovery of genetic variants in β-LG by Aschaffenburg and Drewry (1955), different genetic variants have been found for all major milk proteins. The variants differ from each other in substitution, deletion or addition of amino acids in the primary sequence of the protein. Substitution of amino acids is by far the most common mutation as described in Figure 3.

Due to the amino acids substituted or deleted and also depending on their localization in the protein, the properties of the protein are changed to different degrees regarding net charge, hydrophobicity and/or size. The deletion of 13 amino acids in the A variant of α_{s1}-CN makes it for example less hydrophobic than the B variant. The changes in net charge, hydrophobicity, size, etc., could in addition also change the structure of the casein micelle, the affinity of milk-clotting enzymes, the curd forming properties and the heat stability of the whey

proteins, to mention a few. Taking into account the variation in technical properties of milk due to all known genetic milk protein variants and adding the variation due to pH, temperature, composition, etc., it is easy to understand how difficult it is to predict clotting time, heat stability and other technological properties of a certain milk batch.

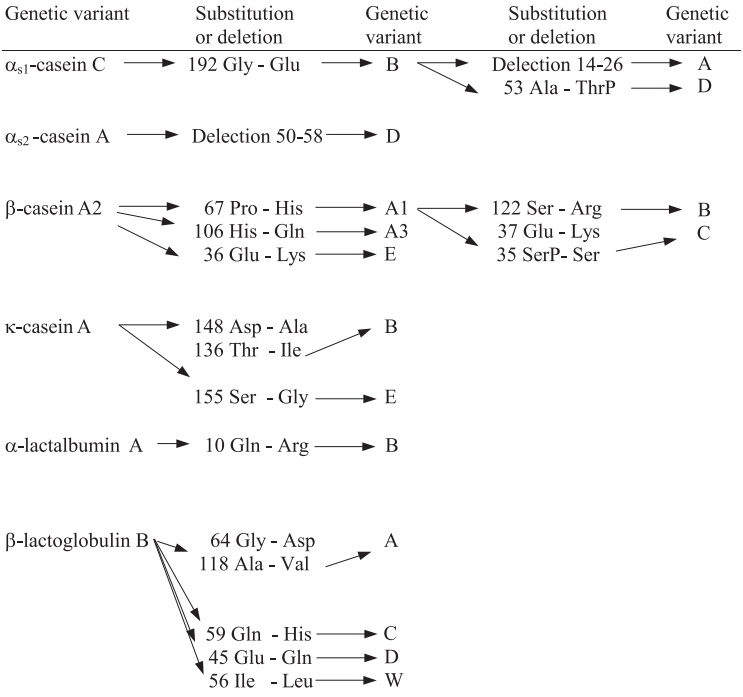

Figure 3. Milk protein genetic polymorphism

Among most breeds, the α_{s2}-CN and α-LA is homozygous for the A and B variant, respectively. Their impact on the variation of the technological properties could therefore be neglected. In Nordic breeds the variants B and C of α_{s1}-CN are found, while the β-CN occurs in the four different variants A^1, A^2, A^3 and B. The variants A and B are the most common of κ-CN, but the E variant is increasing in frequency in Swedish Red-and-White cattle. The latter is due to the use of Finnish Ayrshire breeding bulls, of which the bull Mäkimattilan Inssi, with very good milk production traits, was a carrier of the E allele of κ-CN. The E variant of κ-CN is also difficult to separate from the A variant in gel electrophoretic analyses and was because of that not discovered until 1989 (Erhardt, 1989). Earlier genotyping results based on gel electrophoresis has therefore to be re-evaluated, because of the risk of misinterpreted electrophoretograms. The last major milk protein, β-LG, has the variants A and B in Nordic cattle.

SOME GENERAL AGREEMENTS ON THE EFFECTS OF GENETIC MILK PROTEIN VARIANTS

Considerable research activities have been performed to evaluate influence of different genetic milk protein variants on milk quality and technological properties. However, in addition to variation in the experiments depending on the process studied (see above), variation due to different breeds, lactation stages, feeding regimes, methods of analysis, etc., has to be taken into account. This is also obvious when comparing different studies, because a lot of them give contradicting results.

Nevertheless, some results are generally accepted and are described in the review of Ng-Kwai-Hang (1998). Regarding total protein and casein number (i.e. casein concentration divided with total protein concentration) he stated the following order of α_{s1}-CN genotypes for a higher protein content and casein number: BC>BB>AB. The order of β-CN and κ-CN genotypes for the same parameters was stated to $A^1B>A^2B>A^1A^1>A^2A^2$ and BB>AB>AA, respectively. Since the genotypic order of β-LG for total protein generally is agreed to be AA>AB>BB and depends on a higher expression of the A variant, the order for the casein number will then be BB>AB>AA by definition.

Regarding cheese yield, Ng-Kwai-Hang (1998) did not give any conclusion for the β-CN variants, even if the B variant could be expected to be favourable due to higher casein content in β-CN B milk. For α_{s1}-CN, κ-CN and β-LG the B variant was correlated to a higher yield than the A variant with the genotype AB being intermediate. Wedholm et al. (2006) also found the B variant of β-LG to be correlated to higher cheese yield ($P<0.001$) expressed as g dry cheese solids per 100 g of milk protein.

EFFECTS OF COMBINATIONS OF GENETIC MILK PROTEIN VARIANTS

In many studies the genetic variants of only one of the milk proteins are studied at a time. The drawback in these cases is that any strengthening or counteracting effects of other milk protein variants could be hidden. This is clearly demonstrated by the results of Allmere et al. (2002). When we compared two groups of cows with either κ-CN AA or AE and then further divided the groups according to β-LG variant (AA, AB or BB) the results presented in Table 1 were found. We found curd firmness (gel strength) of chymosin-induced gels to be significantly lower ($P<0.001$) for κ-CN AE samples, while no effect was found for β-LG variants on that parameter. Regarding acid-induced gels on the other hand, the effect of κ-CN variant was much smaller compared to that of β-LG variants. This shows that if the cows had not been genotyped also for the β-LG variant, curd firmness for acid-induced gels could have appeared

Table 1. Relative curd firmness (adjusted for protein concentration) of chymosin- and acid-induced gels of samples containing different genetic variants of κ-CN and β-LG

n	κ-CN	β-LG	Chymosin	Acid
10	AA	AA	34.7	11.8
26	AA	AB	34.7	13.3
14	AA	BB	34.6	31.7
		Weighed average	*34.7*	*14.3*
4	AE	AA	22.1	9.1
5	AE	AB	22.5	11.1
8	AE	BB	21.0	20.5
		Weighed average	*22.0***	*12.4**

favourable for the κ-CN AE genotype if the sampling resulted in only κ-CN AA/β-LG AA versus κ-CN AE/β-LG BB samples. Thus, due to effects of genetic milk protein variants not determined, the influence could differ from study to study. Of course, our results of the κ-CN AE effect on chymosin-induced gels also have to be considered similarly, since we do not have data regarding genetic variants of α_{s1}-CN and β-CN.

From above it follows that it is desirable to genotype for all genetic milk protein variants in order to have reliable results of their effects on different properties. However, this also implies that the number of cows in a study also increases very much in order to have enough individual samples for all possible genotype combinations. In practice only combinations of the most frequent genetic variants within a breed could be studied thoroughly.

In a study by Hallén et al., 2007; effects of κ-CN and β-CN variants on chymosin-induced curd firmness were studied in an experiment including more samples than the one by Allmere et al. (2002). Again the κ-CN AE genotype was found to give lower curd firmness ($P<0.01$), when compared within β-CN A^1A^1 and A^1A^2 genotypes. Of the β-CN variants, the A^2A^2 genotype overall gave lower curd firmness compared to the A^1A^2 genotype ($P<0.001$). Further, within the β-CN A^2A^2 genotype, κ-CN BB was found to yield better curd firmness than the AA genotype ($P<0.05$) with the AB genotype being intermediate. All these results confirm the study by Ikonen et al. (1999), who also found a correlation between κ-CN B and high curd firmness, while the κ-CN E and β-CN A^2A^2 were correlated to low curd firmness.

SELECTION STRATEGIES AND ECONOMICAL RESPONSE

The economical response on different selection strategies to increase the β-LG B variant in New Zealand cattle has been calculated in different breeding models by Harris (1997). From the starting frequency of about 40% of β-LG B

homozygous, he found that it should take 20 years to reach 93%, if only β-LG BB animals were selected in breeding. Selecting both β-LG AB and BB animals resulted in only 60% β-LG B homozygous cows after 20 years of breeding. The economical response for these two strategies was very poor and resulted in an economical net of only NZ$ 68 and 55 per farm, respectively, over a 20 year period taking into account the losses of the quantitative trait (milk fat, total protein, milk volume, liveweight and survival) and the economical benefits of β-LG BB milk (high casein number, high cheese yield).

However, when including the quantitatively traits mentioned above in the selection of β-LG BB animals, the frequency of β-LG B homozygotes after 20 years was 87% and very close to that of breeding for the β-LG BB-parameter only (93%). Most surprising was that the economical response was found to be as high as NZ$ 1173 per farm, i.e. more than 15 times higher than when only selecting for β-LG BB. From this it could be noted that it takes quite a long time to increase the frequency of a certain genetic variant and also that the quantitative traits have to be taken into account in the breeding strategy in order to get the economical response.

CONCLUSIONS

The substitution of a single amino acid can change the technological properties very much due changes in the net charge, hydrophobicity, secondary or tertiary structure of the protein molecule. Different genetic variants of the major milk proteins can both strengthen and counteract each other for a certain property, which makes it important to genotype for as many genetic variants as possible to obtain a reliable result. The latter leads to a need of a large number of cows for sampling in genetic studies. Finally, due to the very long time necessary to increase the frequency of a certain genetic variant in a population, all of its technological properties have to be fully evaluated before a breeding programme is implemented.

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